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Synthesis and anti-tumor evaluation of novel 25-hydroxyprotopanaxadiol analogs incorporating natural amino acids

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ABSTRACT

In the current study, derivatives of 25-hydroxyprotopanaxadiol (25-OH-PPD) were prepared and their *in vitro* anti-tumor activities were tested on six different human tumor cell lines by standard MTT assay. The results showed that combining an ester group combined with the presence of an amino acid moiety led to a 10-fold improved anti-tumor activity. Compound **1c** exhibited the best anti-tumor activity in the *in vitro* assays. Compounds **2c**, **3c**, **4c**, **5c**, **6c** and **8b** showed better anti-tumor activities compared to the parent compound 25-OH-PPD. The current results may provide useful data for researching and developing new anti-cancer agents.

1. Introduction

Ginsenosides and their aglycones have long been used for medical purposes in treatment of cancer, diabetes, heart problems, etc. [1]. There is increasing interest in developing ginseng products as cancer preventive or therapeutic agents [2–4]. The main ginsenosides are glycosides that contain an aglycone with a dammarane skeleton and include protopanaxadiol-type saponins, such as ginsenosides Rb_1, Rb_2, Rb_3, Rc and Rd, and protopanaxatriol-type saponins such as ginsenosides Re and Rg_1, Rg_2, Rh_1. Among the saponin ginsenosides are compounds with a dammarane structure, of which there are two main classes: panaxadiols (PPD) and panaxatriols (PPT). 25-Hydroxyprotopanaxadiol (20(R)-dammarane-3β, 12β, 20, 25-tetrol, 25-OH-PPD, structure shown in Fig. 1), is a promising anti-tumor natural compound isolated from the fruits of *Panax ginseng* [5]. In our previous study, 25-OH-PPD was found to inhibit cancer cell proliferation, cause cell cycle arrest *in vitro* and inhibit the growth of xenograft tumors without obvious host toxicity *in vivo* [5]. Compared with ginsenoside-Rg_3, an agent already being marketed for cancer therapy [6], 25-OH-PPD exhibits a stronger effect on cancer cell growth, which IC_{50} values (20–60 μM) were 5- to 15-fold lower than those of ginsenoside-Rg_3 [7]. Impressively, compared with other ginsenosides, 25-OH-PPD has a higher absolute oral bioavailability and can be obtained in a higher amount [8,9].

Previous studies on the structure–activity relationships (SAR) of ginsenosides suggested that the anti-cancer activities were affected by structural types of dammarane saponin, and numbers of sugar moieties and substituent groups in the side chain of the aglycone also played crucial roles for their anti-cancer activities [10–13]. 25-OCH_3-PPD, a derivative of 25-OH-PPD, has subtle distinction on structure from the latter, but shows better anti-tumor activity. The biological activities of 25-OCH_3-PPD and its known analogs were evaluated in 12 human cancer cell lines. In all cell lines, the order of anti-tumor activities of the test compounds was 25-OCH_3-PPD > 25-OH-PPD > PPD > Rg_3. 25-OCH_3-PPD also induced apoptosis, cell cycle arrest in the G1 phase and inhibited proliferation in breast cancer cell lines, which demonstrating its potent biological effects. The IC_{50} values of 25-OCH_3-PPD for most cell lines were in the lower μM range, a 5- to 15-fold greater cytotoxicity relative to PPD and a 10- to 100-fold increase over Rg_3 [14–16].

Improving the anti-tumor activity of 25-OH-PPD without forfeiting its apoptotic effect is one of the big challenges when dealing with the derivatization process of 25-OH-PPD. Changing the polarity pattern of the molecule is a good option, in which introducing an extra amino group (for gain of polarity) and a protected amino group (for gain of lipophilicity) to 25-OH-PPD. Assuming that the amino acid derivatives should especially benefit the solubility index of these compounds even if esterases cleave the ester prodrug *in vivo* it will still fall apart into the desired triterpane and the

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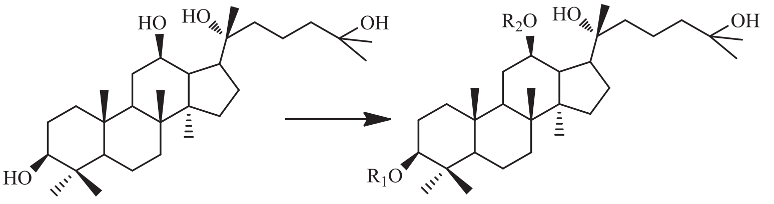


Fig. 1. 25-OH-PPD and its amino acid derivatives.

non-hazardous amino acids. In order to find more potent, higher selectivity compounds, we synthesized 20 derivatives of 25-OH-PPD, esterified for the protection of the amino acids at C(3) and C(12) and varied by coupling with amino acids at C(3), and the structures of these compounds were identified using IR, MS, ^{1}H NMR and ^{13}C NMR spectroscopy. Meanwhile, their biological activities (IC$_{50}$ values) were compared using a MTT assay with six human cancer cell lines including human breast cancer cell line MCF-7, human lung cancer cell line A549, human colorectal cancer cell line Lovo, HCT-116, HT-29 and human glioblastoma U-87 cells. 25-OH-PPD and 5-Fu were used as references.

Table 1
Numbering, structure of the 25-OH-PPD protected and deprotected esters with amino acids.

Compound	R$_1$	R$_2$	Compound	R$_1$	R$_2$
1a	H	Boc-Ala	**4b**	Boc-Pro	H
1b	Boc-Ala	H	**4c**	Pro	H
1c	Ala	H	**5a**	H	Boc-Met
2a	H	Boc-Val	**5b**	Boc-Met	H
2b	Boc-Val	H	**5c**	Met	H
2c	Val	H	**6a**	H	Boc-Phe
3a	H	Boc-Gly	**6b**	Boc-Phe	H
3b	Boc-Gly	H	**6c**	Phe	H
3c	Gly	H	**7b**	Boc-Lys(Boc)	H
4a	H	Boc-Pro	**8b**	Boc-Arg	H

2. Experimental

2.1. Chemistry

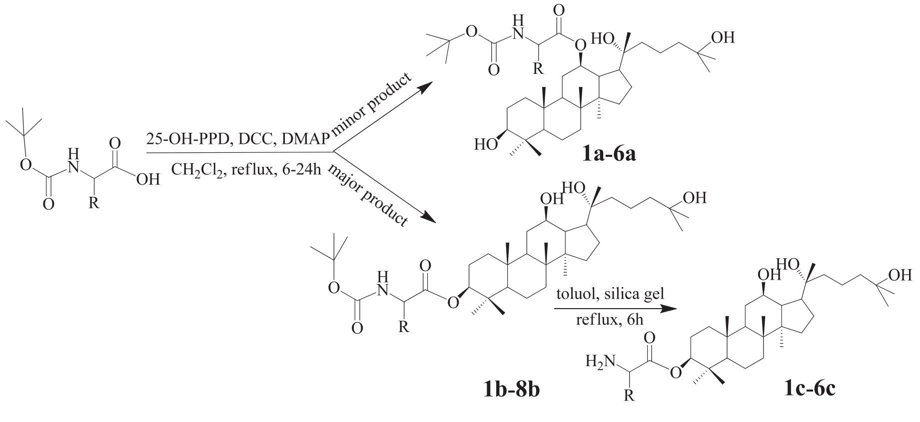
2.1.1. General

The compounds **1a**–**6c** were synthesized by reacting 25-OH-PPD with a series of natural amino acids in the presence of DCC (N,N′-dicyclohexylcarbodiimide) and DMAP (4-dimethylaminopyridine) mediated esterifications of N-Boc protected amino acids (alanine, valine, proline, glycine, methionine, phenylalanine, lysine and arginine) in dry dichloromethane [17–21]. Deprotection of the Boc group was achieved using silica gel catalyzed in toluol for 6 h (Scheme 1 and Table 1). All the products were purified from the reaction mixture by silica gel column chromatography and the structures were confirmed by IR, ^{1}H NMR, ^{13}C NMR and mass spectra. Melting points were determined in open capillary tubes and are uncorrected. IR spectra were recorded (in KBr) on a FT-IR 1730 (PerkineElmer, USA). ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker AV-300 spectrometer using tetramethylsilane as the internal standard. The major chemicals were purchased from Sigma–Aldrich and Fluka.

*2.1.2. General procedure for the protection of the amino acids **1a**–**8b***

The 25-OH-PPD (1 equiv, 46 mg, 0.1 mmol) was added to a solution of DCC (2 equiv, 25 mg), DMAP (2 equiv, 41 mg) and N-Boc-alanine (1 equiv) in dry dichloromethane (50 mL) and the reaction mixtures were shaken for 24 h. The solvent was removed under reduced pressure to give a white solid. The white solid was dissolved in ethyl ether and washed with NaHCO$_3$ (5%), dried (MgSO$_4$) and concentrated under reduced pressure to give the crude product. The crude products were chromatographed using silica gel and eluted with petroleum ether/ethyl acetate (1:2) to give the pure products **1a** and **1b**. Using the same procedure described above for compounds **2a**–**8b**.

*2.1.2.1. (20R)-12β-O-(Boc-L-alanyl)-dammarane-3β, 20, 25-triol (**1a**).* Yield: 11%. Mp: 175–177 °C. ^{1}H NMR (300 Hz, CDCl$_3$): δ 4.72 (1H, td, J = 4.7, 10.4 Hz; H-12), δ 3.47 (1H, dd; H-3), δ 2.30 (2H, q; CH$_2$), δ 1.16 (3H, t; J = 7.5 Hz; CH$_3$), δ 1.22 (6H, s, 2×CH$_3$), δ 1.15 (3H, s, CH$_3$), δ 1.02 (3H, s, CH$_3$), δ 0.98 (3H, s, CH$_3$), δ 0.97 (3H, s, CH$_3$), δ 0.86 (3H, s, CH$_3$), δ 0.77 (3H, s; CH$_3$); ^{13}C NMR (75 MHz, CDCl$_3$): 38.9 (C-1), 27.9 (C-2), 78.7 (C-3), 38.8 (C-4), 55.8 (C-5), 18.2 (C-6), 34.5 (C-7), 39.7 (C-8), 48.7 (C-9), 37.1 (C-10), 28.3 (C-11), 76.5 (C-12), 45.4 (C-13), 52.8 (C-14), 31.7 (C-15), 27.1 (C-16), 50.1 (C-17), 16.2 (C-18), 16.5 (C-19), 73.7 (C-20), 23.1 (C-21), 42.5 (C-22), 17.9 (C-23), 44.4 (C-24), 71.1 (C-25), 29.1 (C-26), 29.4 (C-27), 28.9 (C-28), 15.4 (C-29), 17.6 (C-30), 172.9 (Ala-



Scheme 1. Synthesis of the esters of 25-OH-PPD with amino acids and their subsequent deprotection. R-side chain of amino acid for compounds **1a**–**6c**.